appliedbiosystems

Vaginal Microbiota Profiling Experiments

APPLICATION GUIDE

for use with:

TaqMan® OpenArray™ Plates

QuantStudio™ 12K Flex instrument with OpenArray™ block (AccuFill™ System)

Publication Number MAN0015669

Revision A.0

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A.0	28 June 2016	New document.

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Contents

1 Introduction and workflow overview

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

This guide describes the OpenArray™ plate high-throughput, sample-to-result workflow for vaginal microbiota profiling. The workflow uses:

- OpenArray™ plates with TaqMan® Assays for vaginal microbiota profiling
- QuantStudio™ 12K Flex instrument with OpenArray™ block (AccuFill™ System)

Vaginal microbiota profiling

Microorganism-specific TaqMan® Assays offer a rapid and accurate approach to investigate and monitor vaginal microbiome composition and dynamics.

We offer a collection of qualified TaqMan® Assays (see "TaqMan® Assays for vaginal microbiota profiling" on page 7) that have been optimized for detection of vaginal microbes. The TaqMan® Assays designs and their target sequences have undergone rigorous bioinformatics selection and analysis to ensure maximum strain coverage while minimizing the potential for off-target cross-reactivity. Qualified TaqMan® Assays for vaginal microbiota profiling demonstrate accurate, reproducible performance in multiple rounds of testing for sensitivity and specificity. The assays perform well with DNA isolated from vaginal samples using optimized MagMAX™ DNA Multi-Sample Ultra Kit protocols.

Additional TaqMan® Assays for microbial targets are available from our predesigned assay collection or can be ordered through the Custom TaqMan® Assays Design Tool.

Workflow: TaqMan® vaginal microbiota profiling experiments

Collect vaginal sample using a compatible system or media (see page 12)

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Chapter 3, "Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit"

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Chapter 4, "Prepare and run vaginal microbiota profiling experiments with OpenArray™ plates"

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Chapter 5, "Export and review vaginal microbiota profiling data"

2 Background and tools for assay content selection

TaqMan® Assays

TaqMan® Assays for vaginal microbiota profiling consist of a pair of unlabeled PCR primers and a TaqMan® probe with a FAM™ dye label on the 5' end and minor groove binder (MGB) and nonfluorescent quencher (NFQ) on the 3' end.

For more information about real-time PCR and TaqMan® Assays, visit **thermofisher.com/qpcreducation**.

TaqMan® Assays for vaginal microbiota profiling

OpenArray™ plates can be configured with the following TaqMan® Assays.

For more information about available TaqMan® Assays for vaginal microbiota profiling, visit **www.thermofisher.com/vm**.

Table 1 TaqMan® Assays for vaginal microbiota profiling

Assay ID	Classification	Organism
Ba04646222_s1	Bacteria	*Atopobium vaginae*
Ba04646225_s1	Bacteria	*Bacteroides fragilis*
Ba04646229_s1	Bacteria	*BVAB2*
Ba04646249_s1	Bacteria	*Chlamydia trachomatis*
Ba04646247_s1	Bacteria	*Enterococcus faecalis*
Ba04646242_s1	Bacteria	*Escherichia coli*
Ba04646236_s1	Bacteria	*Gardnerella vaginalis*

Assay ID	Classification	Organism
Ba04646228_s1	Bacteria	*Haemophilus ducreyi*
Ba04646245_s1	Bacteria	*Lactobacillus crispatus*
Ba04646234_s1	Bacteria	*Lactobacillus gasseri*
Ba04646257_s1	Bacteria	*Lactobacillus iners*
Ba04646258_s1	Bacteria	*Lactobacillus jensenii*
Ba04646230_s1	Bacteria	*Megasphera 1*
Ba04646231_s1	Bacteria	*Megasphera 2*
Ba04646235_s1	Bacteria	*Mobiluncus curtisii*
Ba04646246_s1	Bacteria	*Mobiluncus mulieris*
Ba04646251_s1	Bacteria	*Mycoplasma genitalium*
Ba04646255_s1	Bacteria	*Mycoplasma hominis*
Ba04646252_s1	Bacteria	*Neisseria gonorrhoeae*
Ba04646278_s1	Bacteria	*Prevotella bivia*
Ba04646259_s1	Bacteria	*Staphylococcus aureus*
Ba04646276_s1	Bacteria	*Streptococcus agalactiae* (group B)
Ba04646237_s1	Bacteria	*Treponema pallidum* (Syphilis)
Ba04646254_s1	Bacteria	*Ureaplasma urealyticum*
Fn04646233_s1	Fungi	*Candida albicans*
Fn04646244_s1	Fungi	*Candida dubliniensis*
Fn04646240_s1	Fungi	*Candida glabrata*
Fn04646250_s1	Fungi	*Candida krusei*
Fn04646241_s1	Fungi	*Candida lusitaniae*
Fn04646221_s1	Fungi	*Candida parapsilosis*
Fn04646220_s1	Fungi	*Candida tropicalis*
Pr04646256_s1	Protozoa	*Trichomonas vaginalis*
Vi04230116_s1	Virus	HSV1
Vi04646232_s1	Virus	HSV2

(*Optional*) Reference and control assays

The following optional TaqMan® Assays are available as reference or control assays, including prokaryotic 16S rRNA and the human RNase P RPPH1 gene. These assays can be used in relative quantification applications and to assess the adequacy of the human sample.

Table 2 Optional TaqMan® Assays for vaginal microbiota profiling experiments

Assay ID	Target	Application
Ba04930791_s1	Prokaryotic 16S rRNA	Relative quantification/normalization to bacterial DNA
Hs04930436_g1	Human RNase P RPPH1 gene	• Relative quantification/normalization to human DNA • To assess adequacy of the sample

Optional controls

The TaqMan® Vaginal Microbiota Amplification Control contains target sequences for each vaginal microbiota assay as well as control prokaryotic 16S rRNA and human RNase P RPPH1 gene sequences. This amplification control can optionally be included in vaginal microbiota profiling experiments to verify assay performance and to assist with troubleshooting.

For ordering information, email **QuantStudioFrontDesk@lifetech.com**.

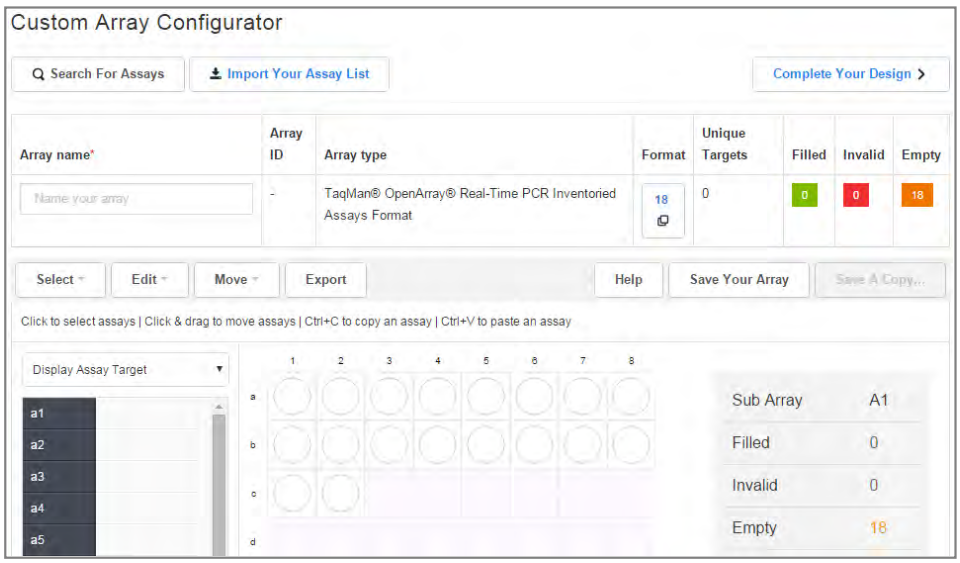
OpenArray™ plate products and formats

TaqMan® OpenArray™ plates contain pre-plated, dried down TaqMan® Assays for vaginal microbiota profiling.

Array format	Number of assays	Number of samples
18	18	48
56	56	48
112	112	24

Configure and order custom TaqMan® OpenArray™ plates

1. Go to **www.thermofisher.com/order/custom-array/**.
2. For array type, select **TaqMan® OpenArray™ Real-Time PCR Inventoried Assays Format**.
3. Click **Select** to configure a plate.



4. Enter the custom array name in the Array name text field.
5. Click **Import Your Assay List**, then provide assay information:
   - Under Upload a list of Assay IDs, click **Choose File**, then select a tab-delimited text file (.txt) containing Assay IDs.

     or
   - Under Enter a list of Assay IDs, paste the Assay IDs, then click **Import Entered List**.
6. Follow the screen instructions to configure the assays on the plate.
7. (*Optional*) Click **Save Your Array** at any time to save the array configuration to your Thermo Fisher Scientific account.
8. When the plate is configured, click **Complete Your Design**, then follow the screen instructions to complete the order.

3 Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit

IMPORTANT! Samples collected using the Hologic™ Aptima™ Vaginal Swab Transport Media (STM) require minor modifications to the DNA isolation procedure (see Appendix B, "Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit: Hologic™ Aptima™ media").

Compatible sample collection systems or media

The following sample collection systems and media are compatible with the procedures described in this guide.

See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.

Sample collection system / media	Source
ThinPrep™ Pap test	Hologic™
BD SurePath™ test	BD™
ESwab™[1,3]	Copan Diagnostics™
Aptima™ Vaginal Swab Transport Media (STM)[2]	Hologic™
M4™ MicroTest™[3]	Remel™
Affirm Ambient Temperature Transport System	BD™
BD ProbeTec™ Swab diluent Q^x	BD™

[1] If samples appear dense or cloudy or have been stored >48 hours, see Appendix A, "Troubleshooting".

[2] Samples require minor modifications to the DNA isolation procedure; see Appendix B, "Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit: Hologic™ Aptima™ media".

[3] Process samples within 48 hours of collection.

Contents and storage

Contents	Cat. No. A25597 (500 rxns)	Cat. No. A25598 (2,500 rxns)	Storage
Proteinase K	4 mL	5 × 4 mL	–25°C to –15°C
PK Buffer	96 mL	5 × 96 mL	15°C to 30°C
Multi-Sample DNA Lysis Buffer	100 mL	5 × 100 mL	
DNA Binding Beads	8 mL	5 × 8 mL	2°C to 8°C
RNase A	2 × 1.25 mL	10 × 1.25 mL	–25°C to –15°C
Nuclease-free Water	100 mL	5 × 100 mL	15°C to 30°C
Wash Solution 1 Concentrate[1]	80 mL	5 × 80 mL	
Wash Solution 2 Concentrate[1]	162 mL	5 × 162 mL	
DNA Elution Buffer 1	25 mL	5 × 25 mL	
DNA Elution Buffer 2	25 mL	5 × 25 mL	

[1] Before use of the kit, prepare all applicable wash solutions as described on their bottles and in this protocol.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Table 3 Required materials and equipment not included with the kit

Item	Source
One of the following instruments	
(Recommended) KingFisher™ Flex Magnetic Particle Processor	5400630
MagMAX™ Express-96 Magnetic Particle Processor	—[1]
Equipment	
Plate shaker, capable of shaking plates at a minimum of 900 rpm	88880023
Analog Vortex Mixer	Fisher Scientific 02-215-365
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(Optional) Magnetic Stand-96	AM10027
Plates and combs	
Deep Well Plates, one of the following:	
KingFisher™ Flex Microtiter Deepwell 96 plates, sterile	95040460
MagMAX™ Express-96 Deep Well Plates	4388476
Standard Well Plates, one of the following:	
KingFisher™ 96 KF microplates	97002540
MagMAX™ Express-96 Standard Plates	4388475
Tip Combs, one of the following:	
KingFisher™ 96 tip comb for DW magnets	97002534
MagMAX™ Express-96 Deep Well Tip Combs	4388487
Other consumables	
MicroAmp™ Clear Adhesive Film	4306311
RNase-free Microfuge Tubes (2.0 mL)	AM12425
Conical tubes (15 mL)	AM12500
Conical tubes (50 mL)	AM12502
Aerosol-resistant pipette tips	MLS

Item	Source
Reagent reservoirs	MLS
(Optional) Paraffin film	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100% (molecular grade or higher)	MLS

[1] Not available for sale.

Table 4 Additional materials and equipment required for processing vaginal samples

Item	Source
Centrifuge, capable of spinning deep-well plates at 2,250 × *g*	Fisher Scientific 75-412-452
Laboratory incubator with slatted shelves, capable of maintaining 65°C	MLS
B-PER™ Bacterial Protein Extraction Reagent	78243
Lysozyme Solution	PI-90082
Zymolyase	Fisher Scientific 50-444-504

Download the KingFisher™ Flex program (if needed)

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor.

1. On the MagMAX™ DNA Multi-Sample Ultra Kit web page, scroll down to the Product Literature section.
2. Click **A25597_Vaginal** to download the program to your computer.
3. See *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. No. N07669) and *BindIt™ Software User Manual* (Cat. No. N07974) for instructions for installing the program on the instrument.

Procedural guidelines

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

- See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.
- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Preheat an incubator to 65°C before each use of the kit.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp™ Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If you use a plate shaker other than the recommended shaker, verify that:
  - The plate fits securely on your plate shaker.
  - The recommended speeds are compatible with your plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.
- To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 5% overage.
- For convenience, you can extend the Proteinase K digestion to 30 minutes.

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
  - Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
  - Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.
- Reconstitute the zymolyase with 500 µL of the provided storage buffer (final concentration of 4 U/µL), vortex to mix, then store at –20°C.

  See the documentation provided with the zymolyase for more information.

Set up the sample layout

Set up the sample plate layout, which provides sample tracking from the 96-well plate used for DNA isolation to the 96-well sample plate *.csv file used for import into the OpenArray™ Sample Tracker Software.

Note: We recommend three technical replicates of each reaction.

Tool	Source	Description
`96-well Sample Plate 1.csv` template	On the computer on which the OpenArray™ Sample Tracker Software is installed: `C:\Program Files\Applied Biosytems\Sample Tracking Utility\examples`	Contains a sample layout tab.

Concentrate the samples

1. Gently shake or swirl the sample contents to ensure thorough mixing of the sample.
2. Following the sample layout, transfer up to 1 mL of sample to the appropriate wells of a deep-well plate.
3. Seal the plate with a clear adhesive film, then centrifuge for 15 minutes at 2,250 × *g* to concentrate the samples.
4. After centrifugation, carefully remove and discard as much supernatant as possible without disturbing the pellet.

   Note: You can leave up to 100 µL of supernatant, especially if there is no pellet.

Digest the samples with the Preliminary Digestion Mix

1. Prepare sufficient Preliminary Digestion Mix according to the following table.

   IMPORTANT! Prepare the Preliminary Digestion Mix no more than 30 minutes before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

Component	Volume per well	Volume per plate
B-PER™ Bacterial Protein Extraction Reagent	185 µL	18.5 mL
Lysozyme Solution	10 µL	1 mL
Zymolyase solution (4 U/µL)	5 µL	0.5 mL
Total Preliminary Digestion Mix	**200 µL**	**20 mL**

2. Add 200 µL of Preliminary Digestion Mix to each sample well.
   (*Optional*) Mix by pipetting up and down 5 to 10 times to disperse large pellets.
3. Seal the plate with a clear adhesive film, then shake for 2 minutes at 1,050 rpm.
4. Incubate the plate for 15 minutes at 65°C.

   IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

   During the incubation, prepare the PK Mix (next section).

Digest the samples with Proteinase K

1. Prepare sufficient PK Mix according to the following table, then invert several times to thoroughly mix components.

   IMPORTANT! Prepare the PK Mix no more than 30 minutes before use and store at room temperature. Do not place PK Buffer or PK Mix on ice, to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	0.8 mL
PK Buffer	42 µL	4.2 mL
Total PK Mix	**50 µL**	**5.0 mL**

2. When the incubation with Preliminary Digestion Mix is complete, add 50 µL of PK Mix to each sample well of the plate.
3. Seal the plate with a clear adhesive film, then shake the sealed plate for 2 minutes at 1,050 rpm.
4. Incubate for 15 minutes at 65°C.

   IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

Set up the processing plates

1. While the samples are incubating at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	5	Standard	DNA Elution Buffer 1	30 µL
Tip Comb	6	Deep Well	Place a tip comb in the plate.	

[1] Position on the instrument

[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1.

2. (*Optional*) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

Add Multi-Sample DNA Lysis Buffer, Bead/RNase A Mix, and isopropanol

1. (*Optional*) If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate for 1–2 minutes at 1,500 × *g*.

2. Prepare sufficient Bead/RNase A Mix according to the following table.

IMPORTANT! Prepare the Bead/RNase A Mix no more than 1 hour before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

Vortex the DNA Binding Beads at moderate speed to form a uniform suspension before preparing the Bead/RNase A Mix.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
RNase A	5 µL	0.5 mL
Nuclease-free Water	19 µL	1.9 mL
Total Bead/RNase A Mix	**40 µL**	**4.0 mL**

3. Add 125 µL of Multi-Sample DNA Lysis Buffer to each sample.

4. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample.

   If you see that the beads in the Bead/RNase A Mix are settling, vortex the mix again briefly before continuing to pipette.

5. Add 200 µL of isopropanol to each sample, then proceed immediately to process the samples on the instrument (next section).

Process samples on the instrument

1. Select the program on the instrument.
   - KingFisher™ Flex Magnetic Particle Processor: **A25597_Vaginal**
   - MagMAX™ Express-96 Magnetic Particle Processor: **4413021_DW_blood**

2. Start the run, remove the temporary paraffin plate seals (if present), then load the prepared processing plates in their positions when prompted by the instrument.

3. Load the sample plate (containing lysate, isopropanol, and Bead/RNase A Mix) at position 1 when prompted by the instrument.

4. When prompted by the instrument (approximately 25 minutes after initial start):
   a. Remove the Elution Plate from the instrument.

   b. Add 30 µL of DNA Elution Buffer 2 to each sample well.

   IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

   c. Load the Elution Plate back onto the instrument, and press **Start**.

5. At the end of the run (approximately 30 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new clear adhesive film.
   - (*Optional*) Eluates can be transferred to a new storage plate after collection.
   - If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.

   IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Prepare and run vaginal microbiota profiling experiments with OpenArray™ plates

This chapter describes how to run profiling experiments with custom OpenArray™ plates using a QuantStudio™ 12K Flex Real-Time PCR System.

This chapter contains brief procedures. For detailed procedures, see the following documentation.

Document	Pub. No.
QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ Sample Tracker Software Quick Reference	4460657
OpenArray™ AccuFill™ System User Guide	4456986

Workflow: Microbiota profiling experiments with OpenArray™ plates

Genomic DNA
(see page 11)

"Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software" on page 23

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"Set up the PCR reactions in an OpenArray™ 384-well Sample Plate" on page 24

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"Set up the AccuFill™ instrument" on page 25

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"Transfer reactions to the OpenArray™ plate (AccuFill™ instrument)" on page 26

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"Seal the OpenArray™ plate" on page 27

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"Run the OpenArray™ plate(s) on the QuantStudio™ 12K Flex instrument" on page 28

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"Check the QC images" on page 29

▼

Chapter 5, "Export and review vaginal microbiota profiling data"

Required materials for OpenArray™ plate workflow

Unless otherwise indicated, all materials are available through **thermofisher.com**.

MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instruments, software, and equipment	
OpenArray™ Sample Tracker Software	—[1]
QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0	A24945
QuantStudio™ 12K Flex instrument with OpenArray™ block (AccuFill™ System)	4471090
Centrifuge, capable of spinning sample plates at 1,000 rpm	MLS
Plates and accessories	
OpenArray™ 384-well Sample Plates, black	4482221
Biomek™ Seal and Sample Foil Lids (for pre-plating step)	Beckman Coulter™ 538619
OpenArray™ AccuFill™ System Tips	4458107
QuantStudio™ 12K Flex OpenArray™ Accessories Kit[2]	4469576
Forceps	MLS
Reagents	
Genomic DNA	See page 11
OpenArray™ plates with TaqMan® Assays	Custom ordered[3]
TaqMan® OpenArray™ Real-Time PCR Master Mix	4462164
Ethanol	MLS

[1] Included with the QuantStudio™ 12K Flex Software.

[2] Each kit contains the items needed to assemble up to 10 plates: 12 lids and plugs, 12 immersion fluid syringes, and 2 carriers. Each custom OpenArray™ plate is shipped with an accessories kit.

[3] See "Configure and order custom TaqMan® OpenArray™ plates" on page 10.

Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software

The software maps samples from the 96-well layout used for DNA isolation to a 384-well sample plate layout as *.csv files that are used by OpenArray™ AccuFill™ software.

See "One-time procedures" on page 30 to:

- Set up optimized folder locations and software preferences before performing this procedure for the first time.
- Download the *.tpf file(s) for the OpenArray™ plate(s) before starting.

1. Generate the 96-well sample plate *.csv file using the 96-Well Sample Plate 1.csv template, then save it to the Sample Tracker 96-well Input folder.

   The 96-Well Sample Plate 1.csv file is provided in the AccuFill™ software installation. Enter or copy the sample names in 96-Well Sample Plate 1.csv, then **Save as** a new *.csv-format file.

   Note: We recommend three technical replicates of each reaction.

2. In the Sample Tracker Software Properties screen, select **Gene Expression** for Experiment Type, then select the appropriate settings for OpenArray™ Plate and Pipettor.

3. In the Samples screen, click  **Import**, then select and import the sample *.csv file.

4. In the Sample Mapping screen, confirm that the samples for a single OpenArray™ plate are assigned to one color.

   If necessary, correct the OpenArray™ Plate and Pipettor settings in the Properties screen.

5. In the Sample Mapping screen, click the **384-Well Plate** tab, then click **Export** ▸ **Export *.csv**.

6. Select **384-Well Plate (for AccuFill)**, then save the exported file.

Plate layouts for the 384-well sample plates are saved to individual *.csv files in the Sample Tracker 384-well CSV Files folder.

Set up the PCR reactions in an OpenArray™ 384-well Sample Plate

IMPORTANT! The 4 × 12 area(s) of the 384-well plate being filled must match the area(s) designated in the OpenArray™ Sample Tracker Software for that set of samples.

1. Remove the OpenArray™ plate from the freezer and allow it to thaw in its sleeve, unopened, at room temperature (~15 minutes).

   The OpenArray™ plate must be completely thawed before transferring reactions to it from the 384-well sample plate.

2. Gently shake the bottle of TaqMan® OpenArray™ Real-Time PCR Master Mix to thoroughly mix the contents. Do not invert the bottle.

3. Following the plate layout designated in the OpenArray™ Sample Tracker Software, add master mix, then DNA samples, to the wells of an OpenArray™ 384-well Sample Plate.

Component	OpenArray™ Plate Format		
	18	56	112[1]
	Volume per well	Volume per well	Volume per well
TaqMan® OpenArray™ Real-Time PCR Master Mix	2.5 µL	2.5 µL	2.5 µL
DNA sample	2.5 µL	2.5 µL	2.5 µL
Total reaction volume	**5.0 µL**	**5.0 µL**	**5.0 µL**

[1] For the 112-format, the OpenArray™ Sample Tracker Software designates two wells for each sample.

4. Seal the plate with an aluminum foil seal, remove the foil flap, mark the edges of the filled 4 × 12 area with a pen, then score the foil along those lines.

   Do not remove the foil from the scored area at this time.

5. Centrifuge the plate for 1 minute at 1,000 rpm.

If you make a sample layout error:

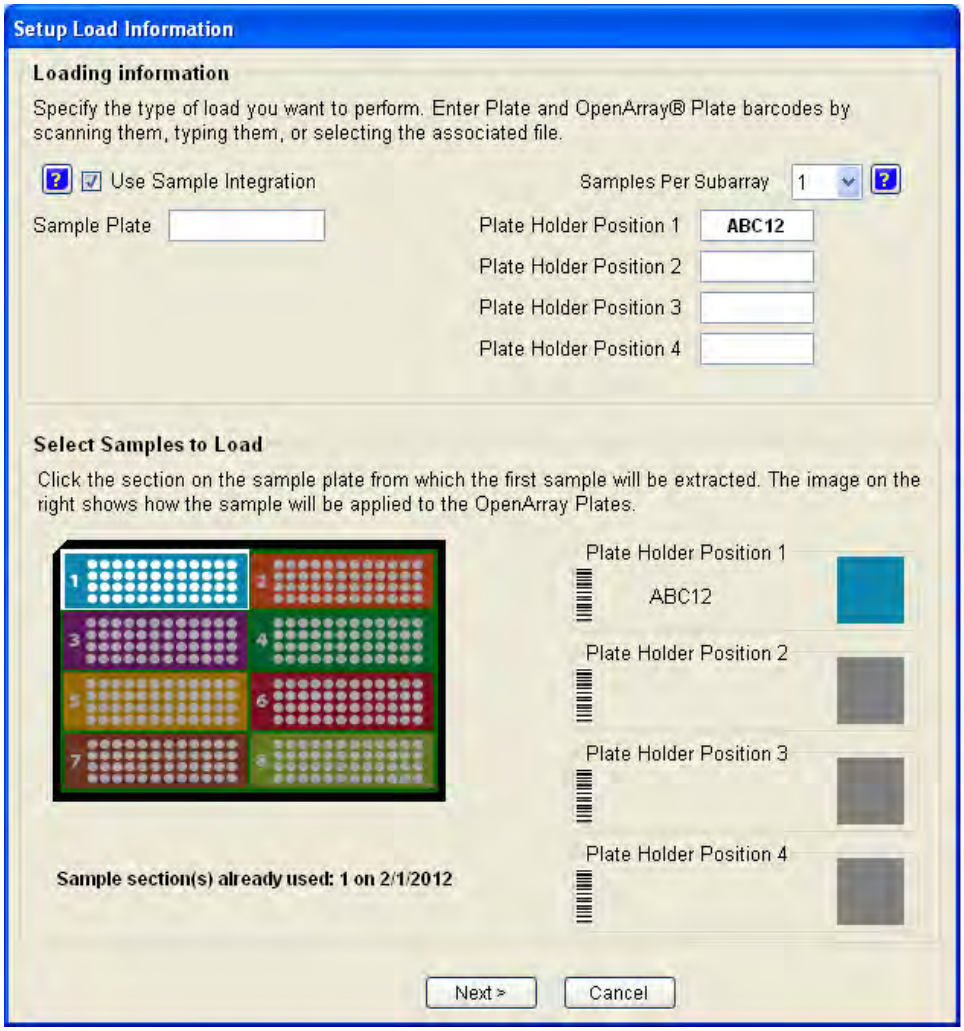
- Before the AccuFill™ procedure – Repeat "Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software" on page 23 with a corrected sample.csv file.
- After the AccuFill™ procedure – See "Recover from layout errors in the 384-well sample plate" on page 30.

Set up the AccuFill™ instrument

IMPORTANT! Before proceeding, check the tip expiration date (shown on the outer box that contains the trays of tips). Do not use tips that exceed the expiration date.

1. In the OpenArray™ AccuFill™ software, click **Setup and Load**.

2. In the Setup Load Information window, verify that the Use Sample Integration checkbox is selected.



3. Click **Browse** to the right of Sample Plate, then select the 384-well sample plate *.csv file that was generated with the OpenArray™ Sample Tracker Software.

4. Click **Browse** to the right of the plate holder position corresponding to the OpenArray™ of interest, then select the *.tpf file corresponding to the desired OpenArray™ plate.

5. Click the corresponding 4 × 12 area of the 384-well plate, then click **Next** to open the Setup Deck window.

6. Ensure that:
   - Tip boxes are loaded in the AccuFill™ instrument in the displayed configuration.
   - Lids are removed from the tip boxes.
   - The waste bin in the instrument is emptied.

7. In the Setup Deck window, select:
   - **The tips are configured as shown above**
   - **The Waste Bin is empty**

Transfer reactions to the OpenArray™ plate (AccuFill™ instrument)

Ensure that the OpenArray™ plate is thoroughly thawed before starting this procedure.

1. Prepare the items needed to seal the OpenArray™ plate (next section).

   Note: The OpenArray™ plate must be sealed promptly after being loaded with the reactions (this section).

   a. Ensure that the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0 is ready.

   b. Gather and remove from packaging an OpenArray™ lid, plug, syringe with OpenArray™ Immersion Fluid, and syringe tip.

   c. Attach the syringe tip to the syringe and carefully push some of the fluid through the tip to remove air bubbles, then lay the syringe aside.

2. Remove the OpenArray™ plate from its sleeve and place it in the plate holder of the AccuFill™ instrument.

   Ensure that the bar code on the OpenArray™ plate is facing left and the serial number is facing right.

3. Using forceps, peel the foil from the filled area of the OpenArray™ 384-well Sample Plate.

4. Close the instrument door.

5. In the AccuFill™ software Setup Deck window, select the following confirmations, then click **Load**.
   - **The OpenArray Plate is in the Plate Holder**
   - **Remove foil from the highlighted section of the Sample Plate**

6. As soon as the Remove OpenArray Plate window appears, open the instrument door, then remove the loaded OpenArray™ plate.

7. Proceed immediately to seal the OpenArray™ plate (next section).

   Note: For best results, seal the OpenArray™ plate within 90 seconds of completion of loading, to prevent evaporation.

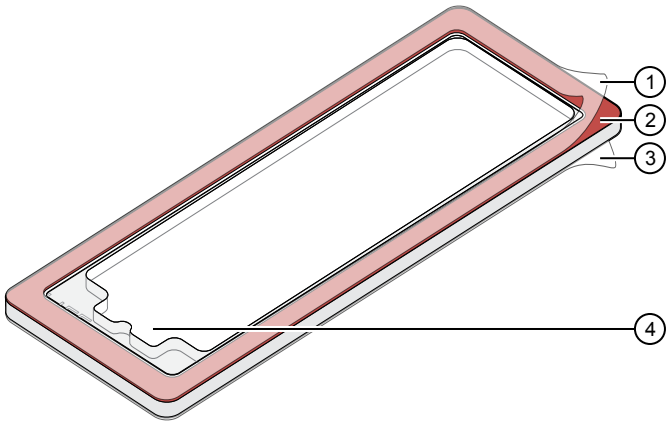
Seal the OpenArray™ plate

IMPORTANT! Handle the OpenArray™ plate and case only by the edges throughout this procedure.

1. Place the filled OpenArray™ plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the bar code is facing left and the serial number is facing right.

2. Remove the clear plastic sheets from the top and the bottom of the lid, remove the red protective film around the edge of the OpenArray™ lid, then seat the lid on the OpenArray™ case in the plate press.



① Protective film (remove)
② Adhesive
③ Protective film (remove)
④ Notched end (align with serial number)

3. Engage the press mechanism until the green flashing light changes to a steady green light (~20 seconds).

4. Disengage the press, then remove the OpenArray™ case.

5. While holding the OpenArray™ case by the edges, insert the prepared syringe tip into the port in the case, then carefully inject immersion fluid until the case is filled.

   Note: Minimize creation of air bubbles when you dispense the fluid; one small air bubble in the case is acceptable.

The syringe tip must be in front of the array when filling the case with immersion fluid.

6. While holding the case vertically, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port and rotate clockwise until the black handle breaks off.

IMPORTANT! To avoid leaking of immersion fluid, hold the case vertically and rotate the plug slowly.

7. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

Run the OpenArray™ plate(s) on the QuantStudio™ 12K Flex instrument

1. On the instrument touchscreen, touch to extend the loading arm, and place the OpenArray™ plates on the plate adapter.

   Ensure that the plate barcode and serial number are facing the front of the instrument.

2. Touch to retract the loading arm.

3. In the Home screen of the QuantStudio™ 12K Flex Software, select **Run ▸ OpenArray**.

4. In the Select Instrument pane, select your QuantStudio™ instrument.

5. Click **Get Plate IDs** to import the barcode(s) of the OpenArray™ plate(s).
   Once the OpenArray™ serial numbers appear, the loaded *.tpf files corresponding to each plate should appear in the Setup File field.
   If not, click **Browse**, then select the correct loaded *.tpf file from the Loaded TPF folder.

6. (*Optional*) Click **Browse** to change the QuantStudio™ Experiment File Location.

7. (*Optional*) Change the software-determined Experiment File Name.

8. Click **Start Run**.

   Note: The instrument pauses at 41 or 42 seconds prior to the end of the run. Wait for the system to complete the run before opening the *.eds file.

9. Transfer the *.eds file from the instrument to an accessible location for analysis.

10. Check the QC images for loading issues or leaks.

Check the QC images

Check the QC images before analysis. For additional information, see Appendix A, "Troubleshooting".

1. In the QuantStudio™ 12K Flex Software  Export screen:
   a. Click **Browse** to create a uniquely-named folder for the QC images export.
   b. Click **Export QC Images** (bottom of screen).

   IMPORTANT! Create a new folder for images each time; exporting a second run to the same folder overwrites the images.

2. View the following ROX™ image to check for loading quality issues:
   - POST-READ_CHANNEL_4.tiff

3. Check for leaks or other displaced sample issues.
   a. View the following spotfinding images:
      - s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff
      - s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff

      Note: The "cp#" in the image file name refers to the array position (1–4) within the instrument.

   b. If a problem is found, view the following pre-run spotfinding image to determine if the issue existed even before cycling (this is useful for troubleshooting):
      - s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff

4. View the following FAM™ images to check for any fluorescent abnormalities and to confirm any problem seen in the spotfinding images:
   - STAGE2_CYCLE1_CHANNEL_1.tiff
   - STAGE2_CYCLE40_CHANNEL_1.tiff

5. Note any abnormalities found, as well as all other potentially relevant information related to the setup of the run.

Recover from layout errors in the 384-well sample plate

After the AccuFill™ procedure, you can recover from plate layout errors that were made during setup of the reactions in the 384-well sample plate. See the *OpenArray™ Sample Tracker Software Quick Reference* (Pub. No. 4460657) for additional information.

1. Create a corrected sample *.csv file.
2. Repeat "Generate 384-well sample plate layouts in the OpenArray™ Sample Tracker Software" on page 23, but select **OpenArray Plate X (for QuantStudio)** when exporting from the OpenArray™ Sample Tracker Software.
3. Import the corrected *.csv file into the QuantStudio™ 12K Flex Software.

   Note: You can import either before starting the run or after the run is complete.

One-time procedures

Set up default folders and software preferences

This procedure simplifies the file locations used in the OpenArray™ AccuFill™ instrument software.

Set up the default file locations and preferences before using the OpenArray™ AccuFill™ system for the first time. You must be logged in as an administrator.

1. Create the following four folders in a convenient location on the same computer drive as the AccuFill™ software:
   - TPF Files
   - Sample Tracker 96-well Input
   - Sample Tracker 384-well CSV Files
   - Loaded TPF Files
2. (*Optional*) Navigate to <*drive*>:`\Program files(x86)\AppliedBiosystems\OpenArray Sample Tracker\Examples`, copy the 96-Well Sample Plate 1.csv file, then paste it in the Sample Tracker 96-well Input folder.
3. In the OpenArray™ Sample Tracker Software, select **View ▸ Preferences**, then enter the following preferences:

Field	Selection
Experiment Type	**Gene Expression**
OpenArray™ Plate	Select the OpenArray™ format that will be run most often; for example, **Gene Expression – 56**
Pipettor	**Fixed** or **Adjustable**
Import Data Directory	**Sample Tracker 96-well Input**
Export Data Directory	**Sample Tracker 384-well CSV Files**

4. In the AccuFill™ software, select **Instrument ▸ Edit Preferences**, then:
   a. Select **Require Sample Integration**.

b. Select the indicated folders.

AccuFill™ folder	Default folder	Folder contents
OpenArray™ Plate File Input Folder	TPF Files	*.tpf files for the OpenArray™ plates; contain assay name and location
Sample Plate File Folder	Sample Tracker 384-well CSV Files	*.csv 384-well sample plate layout files
Loaded OpenArray™ Plate File Folder	Loaded TPF Files	Integrated *.tpf files that are generated during processing with the AccuFill™ software.

5. In the QuantStudio™ 12K Flex instrument software, select **Tools ▸ Preferences ▸ OpenArray**, then select the Loaded TPF Files folder for the software Setup Folder.

   Note: If the instrument software is not on the same computer as the AccuFill™ software, transfer the loaded *.tpf files to the computer running the instrument software.

Download *.tpf files

Set up the optimized folder locations and software preferences before downloading *.tpf files. See "Set up default folders and software preferences" on page 30.

For custom OpenArray™ plates, you need the Lot# and the Serial# from the packaging of each OpenArray™ plate.

1. At **www.thermofisher.com/OA-platefiles**, select **TaqMan® OpenArray™ Custom Gene Expression/Genotyping Plates** in the Select Your Product dropdown list.

2. Select a download option:

   - **I want to download all available TPF & AIF files**
   - **I want to download a specific TPF file**

3. Enter the Lot# and the Serial#, then click **Submit**.

   Note: The Serial# is case-sensitive.

4. Save the *.tpf files to the desktop TPF Files folder.

   Note: Do not create sub-folders in the TPF Files folder. The software cannot access sub-folders.

5 Export and review vaginal microbiota profiling data

Export data

1. Open an *.eds file in the QuantStudio™ 12K Flex Software.
2. In the Experiment Menu pane, click **Export**.
3. Click **Load Export Set** (bottom of the screen), select **GE_export_setting**, then click **OK**.
4. Select **.xlsx** from the File Type dropdown list (top-right of the screen).
5. (*Optional*) Perform any of the following actions to customize the file export:
   - Select **Open file(s) when export is complete**.
   - Click **Browse** to select a new Export File Location.
   - Enter a new file name in the Export File Name text field.
   - Click the **Results** tab, then select the content to export.
6. Click **Start Export** (bottom of the screen).
   If **Open file(s) when export is complete** is selected, then the file automatically opens. If the option is not selected, navigate to and open the exported *.xlsx file.

Prepare exported data for analysis

1. Open the exported *.xlsx data file.
2. Ensure that the barcode, run conditions, and all selected data columns were exported correctly.
3. Scroll down to the data rows, select the headers and data, then copy-paste into a new worksheet.
4. Rename the new worksheet **Data Table_*Run File Name***.

5. (*Optional*) To combine data from multiple OpenArray™ plates:
   a. Insert a Barcode column in the Data Table worksheet to track OpenArray™ barcodes.
   b. Copy-paste the barcode numbers to the appropriate cells in the new Barcode column.
6. Find-replace all "Undetermined" values with an empty cell (no value) in the C_{rt} column.
   This step ensures an exact count of C_{rt} values.
7. Delete rows that do not contain run data.

Review results

Review the exported data for through-hole results that may require special attention.

- Consider omitting through-holes with the following values:

Parameter to examine	Consider omitting through-hole if...
C_{rt}	$C_{rt} \geq 31$
C_q Confidence	C_q Conf < 0.8 **Note:** Possible exceptions could include: • 16s rRNA (Ba04930791_s1) — acceptable range is 0.7 – 1.0 • RNase P (Hs04930436_g1) — acceptable range is 0.7 – 1.0
Amp Score	Amp Score < 1.24 **Note:** Possible exceptions could include: • *G. vaginalis* (Ba04646236_s1)—acceptable range is 1.1 – 1.6

- Take note of technical replicates with mean $C_{rt} \leq 25$ and a high standard deviation (> 0.5). The data from these technical replicates may require further review.
- Ensure that two of the three replicates amplified adequately and passed your review specifications.

  Note: We recommend three technical replicates of each reaction.

Use your preferred method to analyze the data.

Fields for reviewing results with pivot tables

To review results using pivot tables, you can use the following settings.

Note: For the "Average of" and "StdDev of" summarizations, use the appropriate source field (**C_{rt}**, **Amp Score**, or **C_q Conf**), then choose the calculation type.

Area of pivot table	Fields to add	
	Target-oriented view	**Sample-oriented view**
Report Filter	—	Sample Name[1]
Column Labels	Sample Name	—
Row Labels	Target Name	Target Name
Values	Average of C_{rt}	Average of C_{rt}
	StdDev of C_{rt}[2]	StdDev of C_{rt}[2]
	Count of C_{rt}	Count of C_{rt}
	—	Average of Amp Score
	—	Average of C_q Conf

[1] To see individual sample results, select the sample from the dropdown list next to the Sample Name header.

[2] A Values field will automatically appear in the Column Labels area.

A Troubleshooting

Troubleshoot DNA isolation errors

Observation	Possible cause	Recommended action
ESwab™ samples appear cloudy or dense	Samples have been stored for more than 48 hours (at room temperature or lower). **Note:** With extended storage, samples become more viscous which can cause the DNA Binding Beads to clump. DNA recovery may be reduced, impacting downstream performance.	Modify the DNA isolation protocol to improve DNA recovery: 1. Reduce the sample volume to 150–200 µL in "Concentrate the samples" on page 16. 2. Increase the volume of both DNA Elution Buffers to 50 µL in "Set up the processing plates" on page 18.

Troubleshoot with cycling and imaging run images

Many problems with OpenArray™ results can be diagnosed by examining the quality control (QC) images taken at various points during a cycling/imaging run.

The QC images are fluorescent or reflected light images taken before, during, and after cycling. They may require adjustment to make image features visible. To view the images, we recommend that you install the free software program ImageJ, which allows you to easily manipulate the images in ways that other image viewers cannot.

1. In the QuantStudio™ 12K Flex Software  Export screen:
   a. Click **Browse** to select a uniquely-named folder for the QC images export.
   b. Click **Export QC Images** (bottom of screen).

   IMPORTANT! Select a new folder for images each time; exporting a second run to the same folder overwrites the images.

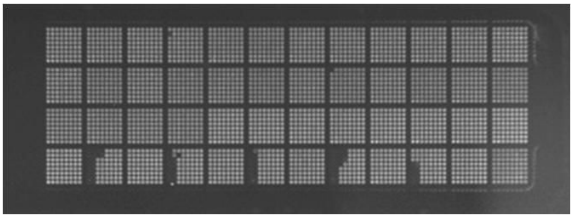
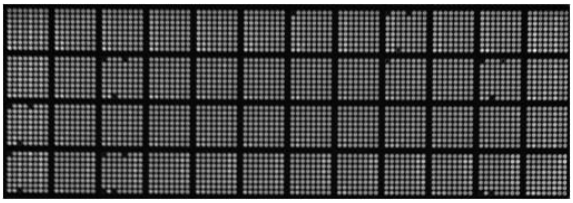
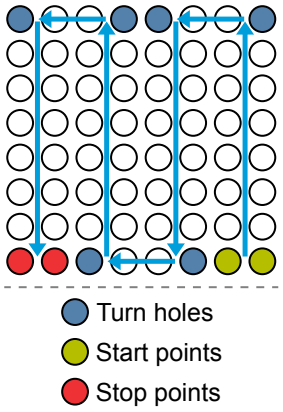
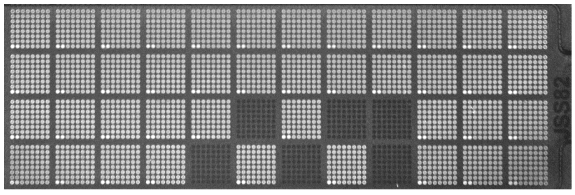
2. Use ImageJ to view the images of interest.

To...	View image...	Image description
Confirm the identity of images within a folder	BARCODE IMAGE.tiff	Reflected light image of the entire OpenArray™ plate
Evaluate the loading quality	PRE-READ_CHANNEL_4.tiff POST-READ_CHANNEL_4.tiff	Pre- and post-ROX™ images
Check for existing contamination on the case and/or heated cover	s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff[1]	Pre-run reflected light spotfinding image (used by the software to determine the location of the holes)
Identify potential leaks or other contamination	s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff[1]	Mid-run reflected light spotfinding image
	s02_c040_t03_p0001_m1_x2_e1_cp#_spotfind.tiff[1]	Post-run reflected light spotfinding image
Look at patterns in the fluorescent data (for example, gradients)	STAGEx_CYCLEy_CHANNEL_1.tiff	FAM™ images at a particular cycle (y) of a particular stage (x) of the run.

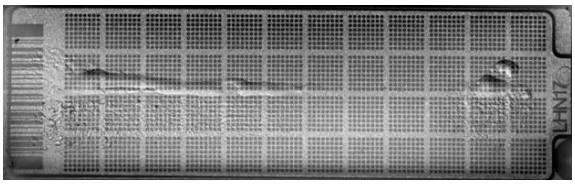
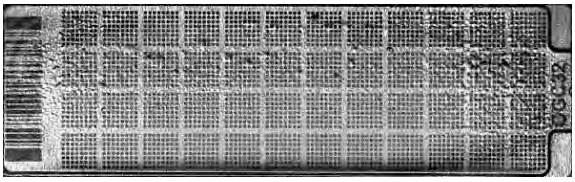
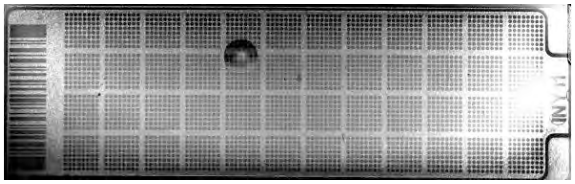
[1] The "cp#" in the image file name refers to the array position (1–4) within the QuantStudio™ 12K Flex instrument.

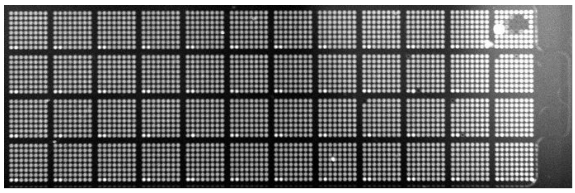
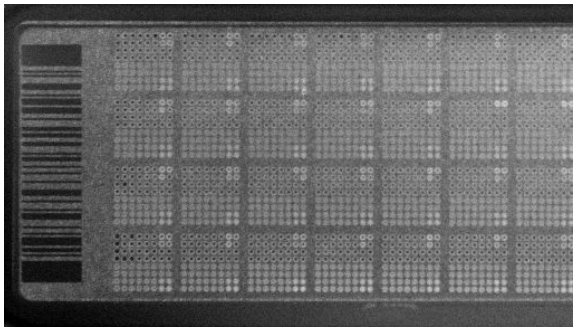
3. (*Optional*) Adjust the images for brightness and/or contrast to make image features visible.
   a. Open the image in ImageJ.
   b. Select **Image ▸ Adjust Brightness/Contrast** (or press **Ctrl+Shift+C**).
   c. Click **Auto** or adjust the sliders until the features of interest in the image are visible.

AccuFill™ instrument plate loading errors

Observation	Possible cause	Recommended action
There are empty through-holes 	Insufficient sample was added to the 384-well sample plate.	Use proper pipetting techniques. Ensure that there are no air bubbles in the pipette tips after sample aspiration.
	Reaction mix (sample + master mix) is not at the bottom of the 384-well sample plate.	Centrifuge the sample plate at 1,000 rpm for 60 seconds.
Turn-holes are repeatedly missed 	AccuFill™ instrument is aligned too far to the left or to the right. Systematic loading problems can occur with the AccuFill™ instrument, which indicates a need for service. For example, when turn-holes are repeatedly missed across multiple subarrays, service is required. Turn-holes are where the AccuFill™ instrument changes direction during sample loading. 	Contact your local field service engineer.
Entire subarrays are missing 	The sample/master mix not added to particular wells in the 384-well sample plate.	Visually inspect the sample plate to ensure that the wells have sample/master mix.
	Stuck tip mandrel on AccuFill™ instrument may need cleaning.	Contact your local field service engineer.
	Pipette tip not loaded on mandrel.	Contact your local field service engineer for frequent occurrences (infrequent occurrences can be due to a poorly molded tip).

OpenArray™ plate assembly and handling errors

Observation	Possible cause	Recommended action
Case leaks and bubbles inside the case    Improper sealing of theOpenArray™ plate in the OpenArray™ Case can lead to immersion fluid leaks or bubble formation inside the case, leading to uneven heating and imaging throughout PCR and to poor quality genotyping data. The images above are examples of OpenArray™ plates that have been affected by immersion fluid leaks. The images show where leaked fluid has condensed on the underside of the heated cover windows and obscured the view of the through-holes. The best image in which to detect leaks is the s02_c001_t03_p0001_m1_x2_e1_cp#_spotfind.tiff image. This image is taken at the start of cycling, which is where most leaks occur. See "Troubleshoot with cycling and imaging run images" on page 36.	Plate press was not engaged for at least 20 seconds.	Fully engage the plate press for at least 20 seconds.
	Damaged lid adhesive.	Remove the liner and visually inspect the lid adhesives for defects. Ensure that adhesive is not damaged or warped.
	Damaged fill port screw gasket.	Visually inspect the screw to ensure that the orange gasket is present and not damaged.
	Damaged fill port screw assembly. Breaks off too easily.	The screw may be mis-threaded: Unscrew it and use a new screw assembly.
	Oily lid or case from immersion fluid overflow.	Wipe off excess overflow of immersion fluid from the lid, case bottom, and crevices with 70% isopropyl alcohol, using a lint-free cloth (the cloth included with the OpenArray™ plate is acceptable).
	Immersion fluid exposed to air for too long.	Do not remove the immersion fluid syringe cap or draw air bubbles into the syringe until you are ready to load. Do not draw air bubbles into the syringe.
	Too large of a bubble inside the OpenArray™ case after sealing.	Minimize the size of the bubble by tilting the OpenArray™ case so that the fill port is at the highest point. Slowly fill the case with immersion fluid until only a small air bubble remains. Attach the screw and wipe off any excess oil that may have spilled onto the case.
	Damaged plate press leading to uneven pressure.	Contact your field service engineer if you suspect that your plate press may be damaged.

Observation	Possible cause	Recommended action
Sample blow out during the addition of immersion fluid 	The reactions in A12 were compromised during the addition of immersion fluid. Injecting the immersion fluid too quickly can actually purge the sample out of the through-holes near the fill port. Often this is caused by the user not purging the syringe slightly before use.	Dispense a small amount of immersion fluid onto a paper towel before use to ensure smooth operation of the syringe.
Evaporation of reaction mixture in through-holes 	Too much time elapsed before plate was sealed with lid and immersion fluid. In this example, the top half of each subarray was intentionally left open to the environment to demonstrate the effect of evaporation. "Donuts" are a result of the evaporated fluid in the though-holes.	Add immersion fluid as soon as the case is removed from the plate press to minimize the likelihood of evaporation, then seal the case with the lid.

B

Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit: Hologic™ Aptima™ media

These are modified instructions specific to samples collected using the Hologic™ Aptima™ Vaginal Swab Transport Media (STM). This modified protocol does not include a preliminary concentration of samples, so increased volumes of PK Buffer and binding reagents are required.

See Chapter 3, "Isolate DNA using the MagMAX™ DNA Multi-Sample Ultra Kit" for information on:

- Kit contents and storage (page 12)
- Materials and equipment required but not supplied (page 13)
- Downloading the KingFisher™ Flex program (page 14)
- Setting up the sample layout (page 16)

See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.

Procedural guidelines

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

- See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.
- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Preheat an incubator to 65°C before each use of the kit.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp™ Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If you use a plate shaker other than the recommended shaker, verify that:
  - The plate fits securely on your plate shaker.
  - The recommended speeds are compatible with your plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.
- To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 5% overage.
- For convenience, you can extend the Proteinase K digestion to 30 minutes.

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
  - Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
  - Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.
- Reconstitute the zymolyase with 500 µL of the provided storage buffer (final concentration of 4 U/µL), vortex to mix, then store at –20°C.

  See the documentation provided with the zymolyase for more information.

Digest the samples with the Preliminary Digestion Mix

1. Prepare sufficient Preliminary Digestion Mix according to the following table.

   IMPORTANT! Prepare the Preliminary Digestion Mix no more than 30 minutes before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

Component	Volume per well	Volume per plate
B-PER™ Bacterial Protein Extraction Reagent	185 µL	18.5 mL
Lysozyme Solution	10 µL	1 mL
Zymolyase solution (4 U/µL)	5 µL	0.5 mL
Total Preliminary Digestion Mix	**200 µL**	**20 mL**

2. Invert the sample collection vial 5 times to ensure thorough mixing of the sample.
3. Following the sample layout, transfer 200 µL of sample to the appropriate wells of a deep-well plate.
4. Add 200 µL of Preliminary Digestion Mix to each sample well.
5. Seal the plate with a clear adhesive film, then shake for 2 minutes at 1,050 rpm.
6. Incubate the plate for 15 minutes at 65°C.

   IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

   During the incubation, prepare the PK Mix (next section).

Digest the samples with Proteinase K

1. Prepare sufficient PK Mix according to the following table, then invert several times to thoroughly mix components.

   IMPORTANT! Prepare the PK Mix no more than 30 minutes before use and store at room temperature. Do not place PK Buffer or PK Mix on ice, to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	0.8 mL
PK Buffer	72 µL	7.2 mL
Total PK Mix	**80 µL**	**8.0 mL**

2. When the incubation with Preliminary Digestion Mix is complete, add 80 µL of PK Mix to each sample well of the plate.

3. Seal the plate with a clear adhesive film, then shake the sealed plate for 2 minutes at 1,050 rpm.

4. Incubate for 15 minutes at 65°C.

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

Set up the processing plates

1. While the samples are incubating at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	5	Standard	DNA Elution Buffer 1	30 µL
Tip Comb	6	Deep Well	Place a tip comb in the plate.	

[1] Position on the instrument

[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1.

2. (*Optional*) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

Add Multi-Sample DNA Lysis Buffer, Bead/RNase A Mix, and isopropanol

1. (*Optional*) If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate for 1–2 minutes at 1,500 × *g*.
2. Prepare sufficient Bead/RNase A Mix according to the following table.

   IMPORTANT! Prepare the Bead/RNase A Mix no more than 1 hour before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

   Vortex the DNA Binding Beads at moderate speed to form a uniform suspension before preparing the Bead/RNase A Mix.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
RNase A	5 µL	0.5 mL
Nuclease-free Water	19 µL	1.9 mL
Total Bead/RNase A Mix	**40 µL**	**4.0 mL**

3. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.
4. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample.

   If you see that the beads in the Bead/RNase A Mix are settling, vortex the mix again briefly before continuing to pipette.
5. Add 315 µL of isopropanol to each sample, then proceed immediately to process the samples on the instrument (next section).

Process samples on the instrument

1. Select the program on the instrument.
   - KingFisher™ Flex Magnetic Particle Processor: **A25597_Vaginal**
   - MagMAX™ Express-96 Magnetic Particle Processor: **4413021_DW_blood**
2. Start the run, remove the temporary paraffin plate seals (if present), then load the prepared processing plates in their positions when prompted by the instrument.
3. Load the sample plate (containing lysate, isopropanol, and Bead/RNase A Mix) at position 1 when prompted by the instrument.
4. When prompted by the instrument (approximately 25 minutes after initial start):
   a. Remove the Elution Plate from the instrument.

b. Add 30 µL of DNA Elution Buffer 2 to each sample well.

IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

c. Load the Elution Plate back onto the instrument, and press **Start**.

5. At the end of the run (approximately 30 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new clear adhesive film.
   - (*Optional*) Eluates can be transferred to a new storage plate after collection.
   - If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication Number
Isolation of DNA for Vaginal Microbiota Profiling Experiments Quick Reference	MAN0015935
OpenArray™ Vaginal Microbiota Profiling Experiments Quick Reference	MAN0015936
QuantStudio™ 12K Flex Real-Time PCR System: OpenArray™ Experiments User Guide	4470935
OpenArray™ Sample Tracker Software Quick Reference	4460657
OpenArray™ AccuFill™ System User Guide	4456986

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- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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